

**MONOCLONAL ANTIBODY SPECIFIC FOR AN EPITOPE OF INACTIVATED
FELINE IMMUNODEFICIENCY-ENCODED GLYCOPROTEIN**

BACKGROUND OF THE INVENTION

5 This application claims priority from copending provisional application serial number 60/410,246, filed on September 12, 2002, the entire disclosure of which is hereby incorporated by reference.

10 Feline immunodeficiency virus (FIV), originally feline T-lymphotropic lentivirus, was first reported by Pederson *et al.*, Science, (1987) 235:790-793 and has been identified in domestic cats and cheetahs. The infection is endemic in cats throughout the world. Like HIV, FIV is an international concern. According to the American Association of Feline Practitioners, up to one in twelve cats may test positive for FIV. After infection, there is a transient period of fever, lymphadenopathy and
15 neutropenia. Most cats recover from this stage and appear normal for months or years before immunodeficiency occurs. Due to this latent manifestation of immunodeficiency, it would be unduly hazardous to utilize a live virus vaccine for the treatment or prevention of FIV. Although monoclonal antibodies specific for epitopes of FIV-encoded antigens or antigenic proteins are known, i.e. US 5,177,014 and US
20 5,219,725, these antibodies are not capable of recognizing inactivated FIV. This means that for current, commercial FIV vaccines, all of which utilize inactivated FIV, there are no known monoclonal antibodies useful for the determination of virus quantity or the potency of the inactivated FIV component in a vaccine composition.

25 Therefore, it is an object of this invention to provide a monoclonal antibody specific for an epitope of an inactivated FIV glycoprotein.

 It is another object of this invention to provide a method for the determination of the quantity of an inactivated FIV.

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 It is a further object of this invention to provide a method to determine the potency of an inactivated FIV vaccine.

It is a feature of this invention that the monoclonal antibody of the invention is specific for the epitope of an inactivated FIV glycoprotein and does not recognize epitopes of live FIV glycoproteins, proteins or antigens.

- 5 Further objects and features of the invention will become more apparent from the detailed description set forth herein below.

SUMMARY OF THE INVENTION

- 10 The present invention provides a monoclonal antibody specific for an epitope unique to an inactivated feline immunodeficiency virus-encoded glycoprotein.

- The present invention further provides a method for the detection of an epitope unique to an inactivated feline immunodeficiency virus-encoded glycoprotein in a sample which comprises: contacting said sample with a monoclonal antibody
15 specific for an epitope unique to an inactivated feline immunodeficiency virus-encoded glycoprotein to form a complex; and detecting said complex.

BRIEF DESCRIPTION OF THE DRAWING

- 20 FIG. 1 is a photograph of a Western immunoblot analysis of the monoclonal antibody, mAb 1D9, identified as specific for a unique epitope of an inactivated feline immunodeficiency virus-encoded glycoprotein.

- FIG 1 is further described as follows: Immunoprecipitation of FIV envelope glycoproteins with monoclonal antibody 1D9. A virus stock enriched in formalin-inactivated FIV was biotinylated with sulfo-NHS-LC-biotin, and extracted with Triton
25 X-100. Immunoprecipitation was carried out by incubating the extract with mAb 1D9 or H5332. The immune complexes were collected on Immobilized Protein G, washed and subjected to SDS-PAGE and Western blotting. The proteins on the blot were detected using peroxidase-labeled streptavidin. Lanes 1 and 5, biotinylated
30 molecular weight marker; lane 2, 20 uL of mAb 1D9 used for immunoprecipitation; lane 3, 100 uL of 1D9 used for immunoprecipitation; lane 4, 100 uL of an irrelevant monoclonal antibody, H5332, used in immunoprecipitation. The H (50Kd) and L

chains (25 Kd) of the antibodies and some BSA (67 Kd) were non-specifically stained by peroxidase-labeled streptavidin.

DETAILED DESCRIPTION OF THE INVENTION

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The feline immunodeficiency virus (FIV) envelope glycoprotein is involved in receptor interactions with cells and this interaction determines the cells susceptibility to said virus. The FIV envelope glycoprotein is also involved in virus penetration and syncytium formation and is the primary target for humoral and cellular immune responses. (Bendinelli, M., *et al.*, Clinical Microbiology Review, (1995) 8:87-112) Said envelope glycoprotein consists of two components which are non-covalently bound in the virion, the surface (SU) protein which is heavily glycosylated and has an apparent molecular weight of 95,000-100,000 and the trans-membrane (TM) protein which is less glycosylated and has an apparent molecular weight of 35,000-40,000. (Pancino, G. *et al.*, Virology, (1995) 206:796-806). Various studies have suggested that the FIV envelope protein is capable of inducing protective FIV immunity in cats. Therefore, an enzyme-linked immunosorbent assay (ELISA) which measures the relative quantity or purity of the FIV envelope protein would be most useful for determining the potency of an FIV vaccine. However, all current commercially available FIV vaccines utilize inactivated or killed virus and the monoclonal antibodies known to react with or recognize FIV or FIV glycoprotein do not react with or recognize inactivated FIV or FIV glycoprotein. It is proposed that the inactivation of said virus may alter the conformation of the FIV envelope protein.

Surprisingly, it has now been found that the monoclonal antibody, designated mAb 1D9, specifically recognizes only inactivated FIV and does not recognize live FIV. Further, ELISA and immunoprecipitation experiments demonstrate that the monoclonal antibody of the invention reacts specifically with the surface protein component of the FIV envelope glycoprotein.

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The term monoclonal antibody, as used in the specification and claims, designates an antibody produced from a single antibody-producing cell which has been cloned to produce an antibody-producing cell line. The term epitope designates

a specific amino acid sequence, modified amino acid sequence, or protein secondary or tertiary structure which is recognized by an antibody. The term inactivated virus designates "not live" or "killed" virus.

5 The monoclonal antibody of the invention may be prepared using conventional techniques known in the art. For example, mice may be immunized with a partially purified, inactivated virus such as FIV-Shiz, FIV-Petaluma, or the like, preferably FIV-Shiz; mouse tail bleeds are then screened for antibody response and selected for fusion; cloned hybridoma cells are then selected and screened for
10 specific reactivity with inactivated FIV. The hybridoma for the single stable clone thus obtained may be grown in a bioreactor and multiple harvests of the antibody may be pooled to generate the desired monoclonal antibody, mAb 1D9.

 Said antibody may be produced by a cell line deposited with the American
15 Type Culture Collection and assigned the number ATCC number PTA-4837.

 Inactivation of the virus may be achieved by conventional inactivating means, for example chemical inactivation using chemical inactivating agents such as binary ethyleneimine, phenol, α -lactopropionate, beta-propiolactone, formalin, merthiolate,
20 glutaraldehyde, sodium dodecyl sulfate, or the like or a mixture thereof, preferably formalin. Said virus may also be inactivated by heat or psoralen in the presence of ultraviolet light.

 The monoclonal antibody of the invention is specific for inactivated FIV and
25 forms a sufficiently strong interaction with an epitope unique to an inactivated FIV envelope glycoprotein, such as gp 95 or gp 130, to be useful in an assay for the determination of the quantity of an inactivated virus or for the potency of an inactivated FIV vaccine. Accordingly, the present invention provides a method for the detection of an epitope unique to an inactivated FIV-encoded glycoprotein in a
30 sample which comprises: contacting said sample with a monoclonal antibody specific for an epitope unique to an inactivated FIV-encoded glycoprotein to form a complex; and detecting said complex.

Samples suitable for use in the method of the invention include those which contain an inactivated virus or inactivated virus-infected cells in a culture medium or in a vaccine composition.

5 Means of detecting the complex suitable for use in the method of the invention include any conventional means generally used to detect monoclonal antibody protein complexes such as detection by enzyme-, fluorochrome-, or biotin-labeled anti-mouse antibody, detection by Protein A, or the like. In actual practice, the method of the invention may be implemented in the form of an ELISA or
10 immunoprecipitation assay having the monoclonal antibody, mAb 1D9, as the detection antibody.

For a more clear understanding of the invention, the following examples are set forth below. These examples are merely illustrative and are not understood to limit
15 the scope or underlying principles of the invention in any way. Indeed, various modifications of the invention, in addition to those shown and described herein, will become apparent to those skilled in the art from the examples set forth hereinbelow and the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

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Unless otherwise noted, all parts are parts by weight.

EXAMPLE 1

Preparation of a Monoclonal Antibody Specific for an Epitope of an Inactivated
25 **FIV-encoded Glycoprotein**

Cells and Viruses

FIV-Shizuoka (FIV-Shiz, a subtype D FIV) was propagated in persistently infected lymphoid cell lines, derived from FIV-Shizuoka and FeT-J (ATCC Accession
30 No. CRL 11967), an IL-2 independent cell line, designated Shiz. An FIV-Shizuoka persistently infected cell line is also deposited at ATCC under Accession No. CRL 11976. To generate antigen stocks, virus fluids were inactivated using formalin and concentrated using ultrafiltration.

Antigen stocks are also similarly prepared from a variety of other FIV strains and subtypes, such as, field isolates, FIV strain NCSU 1 (ATCC Accession No. VR-2333), FIV strain UC24818 (ATCC Accession No. VR-2619), FIV-Petaluma (subtype A, ATCC Accession No. VR-2186), FIV-Dixon (subtype A), FIV-UK8 (subtype A), FIV-Bangston (subtype B), FIV-Amori-1 (subtype B), FIV-Amori-2 (subtype B), propagated on appropriately susceptible cell lines and IL-2 dependent or independent feline T-cell lines, such as, PMBCs, CRFK, NYA-1 (ATCC Accession No. CRL-2417), FeT-1M (ATCC Accession No. CRL-10775), FeT-2D (ATCC Accession No. 10774), Fet-1C (ATCC Accession No. CRL-11968), FL-4 (ATCC Accession No. 10772), FL-6 (ATCC Accession No. 10773), or propagated on FIV persistently infected cell lines derived therefrom, such as, the FIV-CRFK cell line having ATCC Accession No. VR-1312, the FIV-Bangston infected cell line deposited under ATCC Accession No. 11975, and the like, such as those disclosed, for example, in U.S. Patent No. 6,254,872.

GENERATION OF MONOCLONAL ANTIBODY

Balb/c mice were immunized twice with formalin-treated FIV-Shiz virus which is purified using glycerol gradient technique. The injection site was subcutaneous for both injections. Mouse tail bleeds are screened for antibody response. One mouse which displayed high FIV-specific antibody titers was chosen for fusion. Splenocytes collected from this mouse were fused to SP2/0 myeloma cells. Hybridoma cells were selected as described in "Antibodies: A Laboratory Manual" by Ed Harlow and David Lane for Cold Spring Harbor Press. Primary hybridoma clones were screened for specific reactivity with formalin-treated FIV-Shiz. One stable clone, mAb 1D9, was obtained. The hybridoma for mAb 1D9 was grown in a Heraeus miniPERM bioreactor. Multiple harvests of the antibody were obtained and pooled to generate a large quantity of mAb 1D9.

EXAMPLE 2**Use of Monoclonal Antibody, mAb 1D9, as a Detection Antibody in an Enzyme-Linked Immunosorbent Assay**

5 In this evaluation, Galanthus Nivalis Agglutinin (GNA) is used to capture the glycoproteins. This GNA ELISA combines the high selectivity of GNA binding with its broad reactivity with the glycoproteins of HIV-1, HIV-2, SIV and FIV. To begin, ninety-six microwell plates were coated with 10 µg/mL of GNA in 50 mM carbonate, pH 9.6 for 1 h at 37°C. After blocking wells with PBS-10% FBS for 2 h at 37°C,
10 samples which were treated with 1% Empigen BB (Calbiochem) for 1 h at 37°C were added, and incubated for 2 hours at 37°C. Unbound antigens were removed by washing 3 times with PBS containing 0.1% Tween 20. The monoclonal antibody of Example 1, mAb 1D9 diluted at 1:8,000 was added to each well and the plate was incubated at 37°C for 1 h. After washing, peroxidase-labeled goat anti-mouse IgG,
15 Kirkegaard & Perry Laboratories (KPL), diluted 1:1,000 was added and the plate was incubated at 37°C for 1 h, and then washed and developed with TMB peroxidase substrate (KPL). The plate was read at 650 nm minus 490 nm after a 5 min reaction period.

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EXAMPLE 3**Use of Monoclonal Antibody, mAb 1D9, as a Detection Antibody in an Immunoprecipitation Assay**

25 In this evaluation, a virus stock enriched for the formalin inactivated FIV-Petaluma virus (0.38 mg total protein with less than 5% being FIV proteins) was incubated with 5 mg of sulfo-NHS-LC-biotin (Pierce) in 2 mL of PBS on ice for 1 h. After removing the unincorporated biotin reagent by dialysis, the virus-containing sample was extracted for 1 h with 1% Triton X-100 in 12 mL of PBS, and centrifuged at 100,000 g for 2 h. The supernatant was recovered and used for immunoprecipitation.
30 Immunoprecipitation was carried out by incubating 600 µL of the extract with 80 µL of either mAb 1D9 or mAb H5332 at 4°C for 1 h. The mAb H5332, which has a specificity for Borrelia OspA protein, was used as the irrelevant antibody control. The immune complexes were collected on Immobilized Protein G (Pierce), washed 4

times with cold PBS-1% NP-40, resuspended in Laemmli buffer and subjected to SDS-PAGE and Western blotting. The blot was blocked for 60 min with SuperBlock (Pierce) and then incubated for 45 min with peroxidase-labeled streptavidin (KPL), diluted 1:400,000. The membrane was washed 4 times with PBS-0.05% Tween-20, and the biotin-streptavidin complexes were detected with the SuperSignal chemilluminescence detection kit (Pierce) followed by exposure to X-ray film.

EXAMPLE 4

10 Evaluation of the Specificity of the Monoclonal Antibody, mAb 1D9

CELLS AND VIRUSES

FIV-Shizuoka (FIV-Shiz) and FIV-Petaluma were propagated in persistently infected lymphoid cell lines designated Shiz and FL-6, respectively. Feline leukemia virus (FeLV) was propagated in a chronically infected cell line. Feline calicivirus (FCV), feline viral rhinotracheitis virus (FVR) and feline panleukopenia virus (FPV) were grown on Crandell feline kidney cells. To generate antigen stocks, virus fluids were inactivated with formalin and concentrated using ultrafiltration.

Evaluation

In this evaluation, the specificity of mAb 1D9 was determined using both of the ELISA and immunoprecipitation techniques described hereinabove in Examples 2 and 3.

A - GNA ELISA

Various antigen samples were tested using the assay described in Example 2. The results are shown in Table I below.

Table I

Antigen Sample	Antigen Sample Concentration	Optical Density $A_{650}-A_{492}$ Value
FIV-Shiz virus, inactivated	1x	0.572
FIV-Petaluma virus inactivated	1x	0.385
FIV-Shiz virus, live	1x	<u>0.006</u>
FIV-Petaluma virus, live	1x	<u>0.006</u>
FetJ TCS, inactivated	1x	0.027
FeLV, inactivated	1x	0.020
FCV, inactivated	1x	0.027
FVR, inactivated	1x	0.027
FPV, inactivated	1x	0.026
FeLV, live	$10^{6.63}$ TCID ₅₀ /mL	0.039
FCV, live	$10^{7.67}$ TCID ₅₀ /mL	0.035
FVR, live	$10^{7.46}$ TCID ₅₀ /mL	0.039
FPV, live	$10^{6.75}$ TCID ₅₀ /mL	0.051
No antigen control	0	0.033

Observations

When used at 1:8000 dilution, mAb 1D9 reacted well with both inactivated FIV-Shiz and inactivated FIV-Petaluma samples. In contrast, mAb 1D9 showed no reaction when it was tested with samples of either live FeLV, FCV, FVR and FPV or inactivated antigen stocks for those various viruses. Advantageously, mAb 1D9 does not react with samples of live FIV-Petaluma or live FIV-Shizuoka even though it reacts well with samples of both inactivated FIV-Shiz and inactivated FIV-Shiz. The ELISA data in Table I indicated that the GNA ELISA based on the monoclonal antibody mAb 1D9 may be used to detect specifically the FIV glycoprotein. The observation that mAb 1D9 reacted with formalin-inactivated FIV, not live FIV, indicates that the epitope recognized by mAb 1D9 is a unique epitope created by the formalin treatment of FIV.

B – Immunoprecipitation

To further confirm the specificity of mAb 1D9 for the inactivated FIV envelope glycoprotein, an inactivated FIV-enriched stock was biotinylated and immunoprecipitated as described in Example 3 with mAb 1D9 or mAb H5332, an irrelevant monoclonal antibody. As shown in Figure 1, mAb 1D9 reacted specifically with the SU protein, as indicated by the broad 95-100 Kd band. The band with a higher molecular weight (ca. 160 Kd) might be a complex of SU with another protein which was cross-linked by formalin treatment.

Conclusion

The results of ELISA and immunoprecipitation experiments demonstrated that the monoclonal antibody mAb 1D9 reacted specifically with the surface protein component of the FIV envelope glycoprotein, and is suitable for use in a potency test for inactivated FIV vaccines or for the determination of the quantity of an inactivated virus sample or an inactivated virus-infected cell sample.

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